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<b>(54) Title:</b> PAIN REDUCING PARENTERAL LIPOsome FORMULATION		
<b>(57) Abstract</b> <p>Disclosed is an invention directed towards pain-reducing parenteral formulations comprising a macrolide drug entrapped in a liposome vesicle. The macrolide drug is selected from the group consisting of derivatives of erythromycins A, B, C and D; clarithromycin; azithromycin; dirithromycin; josamycin; midecamycin; kitasamycin; roxithromycin; rokitamycin; oleandomycin; miocamycin; flurithromycin; rosaramicin; 8,9-anhydro-4'-deoxy-3'-N-desmethyl-3'-N-ethylerythronycin B 6,9-hemiacetal; 8,9-anhydro-4'-deoxy-3'-N-desmethyl-3'-N-ethylerythronycin A 6,9-hemiacetal; and 11-amino-11-deoxy-3-oxo-5-O-desosaminyl-6-O-[1'-3'-quinolyl-2'-propenyl]-erythronolide A 11,12-cyclic carbamate. The formulations of the invention are effective in substantially reducing the pain at the injection site typically associated with the injection of macrolide antibiotics.</p>		

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## PAIN REDUCING PARENTERAL LIPOSOME FORMULATION

### Technical Field

The present invention relates to pain reducing parenteral formulation in liposome system. More particularly, it relates to a parenteral formulation comprising a macrolide drug entrapped in a liposome vesicle. The formulations of the invention are effective in substantially  
5 reducing the pain typically associated with the injection of macrolide antibiotics.

### Background Of The Invention

Injection site pain following a parenteral administration is generally caused by pharmacological/physiological reactions between drug molecules and the local tissue. Pain reducing formulations in the form of either micelles or emulsions for intravenous administration of macrolide antibiotics have been disclosed by Lovell, *et al.*, *International Journal of Pharmaceuticals*, 109, 45-57, (1994); Cannon, *et al.*, *International Journal of Pharmaceutics*, 114, 65-74, (1995); and Klement, *et al.*, *British Journal of Anesthesia*, 67, 281-284, (1991).

For emulsion systems, the drugs are entrapped in small oil droplets. Emulsions are generally stabilized by some surfactants which create an interfacial layer to separate oil phase and the aqueous phase. This interfacial layer is a barrier to reduce direct contact of the drug molecules to the local tissue around the injection site. However, this barrier is very dynamic. Drug molecules travel in and out of the interface very frequently. This property limits the effectiveness of emulsion on injection pain reduction.

For the micellar systems, drugs are entrapped in micelles consisting of surfactants. Polar head groups of the surfactant molecules face the aqueous bulk solution and the lipophilic tails face toward the core to form spherical or cylindrical structures. The packing of the head groups is generally not tight and is very dynamic allowing rapid release of the drug following an injection. Similarly, the effectiveness of the micelles in reducing injection site pain is  
25 limited.

Currently, some of these antibiotics, for example clarithromycin, are sold only in the oral form in the United States because of their highly painful and irritative nature at the site of injection. Therefore, there exists a need for formulations which can be used for the injection site pain producing drugs and which are more effective in reducing such pain than the emulsions and micelles known in the art.

### Brief Description Of The Drawings

Figure 1 compares the response to pain by successive infusion of saline solution, clarithromycin solution and saline solution using Rat Tail Vein Infusion Pain Model.

Figure 2 compares the response to pain by successive infusion of saline, placebo liposome, saline, clarithromycin liposome formulation of the invention containing phosphatidylglycerol, saline and clarithromycin solution using Rat Tail Vein Infusion Pain Model.

5 Figure 3 compares the response to pain by successive infusion of saline, placebo liposome, saline, clarithromycin liposome formulation of the invention containing dimyristoyl phosphatidic acid, saline, clarithromycin solution and saline using Rat Tail Vein Infusion Pain Model.

Figure 4 compares the response to pain by successive infusion of saline, placebo liposome, saline, ABT-229 liposome formulation of Example 3 of the invention, saline, placebo 10 IV (lactobionic acid solution), saline, ABT-229 solution IV, and saline using Rat Tail Vein Infusion Pain Model.

Figure 5 compares the response to pain by successive infusion of saline, placebo liposome, saline, ABT-773 liposome formulation of Example 4 of the invention, saline, placebo 15 V (lactobionic acid solution), saline, ABT-773 solution V, and saline using Rat Tail Vein Infusion Pain Model.

#### Summary Of The Invention

In one aspect, the present invention is directed towards a pain-reducing parenteral 20 formulation comprising a macrolide drug entrapped in a liposome vesicle comprising a lipid.

The formulations of the invention are effective on injection site pain reduction caused by macrolide drug molecules.

In another aspect, the present invention is directed to a method of reducing injection pain site caused by a macrolide drug comprising administering a parenteral formulation comprising a 25 macrolide drug entrapped in a liposome vesicle.

#### Detailed Description Of The Invention

The term "liposome vesicle" as used herein refers to a single bilayer or multiple bilayers vesicles consisting of phospholipids or other lipids in which an aqueous volume is entirely 30 enclosed by a membrane composed of lipid molecules.

The parenteral formulations of the invention comprise a macrolide drug entrapped in liposome vesicles which comprise a lipid. The macrolide drugs can be selected from derivatives of erythromycins A, B, C, and D; clarithromycin; azithromycin; dirithromycin; josamycin; midecamycin; kitasamycin; roxithromycin; rokitamycin; oleandomycin; miocamycin; 35 flurithromycin; rosaramycin; 8,9-anhydro-4"-deoxy-3'-N-desmethyl-3'-N-ethylerythromycin B, 6,9-hemiacetal (ABT-229); 8,9-anhydro-4"-deoxy-3'-N-desmethyl-3'-N-ethylerythromycin A, 6,9-hemiacetal (ABT-269); 11-amino-11-deoxy-3-oxo-5-O-desosaminyl-6-O-[1'-3'-quinolyl]-

2'-propenyl]-erythronolide A 11,12-cyclic carbamate (ABT-773); and the like. Preferably, the macrolide drug used in the formulation is clarithromycin, ABT-229, and ABT-773. More preferably, the macrolide drug used in the formulation is clarithromycin.

The liposome vesicle is formed when lipids are dispersed in an aqueous medium.

- 5 Exemplary, but not limiting, of lipids that can be used in forming the liposomes are naturally occurring lipids, synthetic lipids and semi-synthetic lipids. The naturally occurring lipids include phospholipids, phosphatidyl choline, fatty acids, double chain secondary amines and cholesterol derivatives, and the like. Synthetically obtained lipids include, but are not limited to, ether-linked phosphatidyl choline, dimyristyl lecithin, dipalmitoyl lecithin, distearoyl lecithin. Preferably, the lipids are phospholipids which include phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl-inositol, phosphatidyl serine, sphingomyelin, ether-linked phosphatidyl choline, dimyristoyl phosphatidyl choline, dimyristoyl phosphatidic acid, dimyristoyl phosphatidyl glycerol, phosphatidic acid and the like. The molar ratio of the drug molecules to the lipid can be as low as 1:2 or may contain a higher proportion of lipid such as 1:100. Preferably, this molar ratio varies from 1:8 to 1:20. The molar ratio depends upon the drug being used but will assure the presence of a sufficient number of neutral and/or negatively charged lipids and the drug to form a stable complex with most drugs. Preferably, the liposome vesicles comprise both the neutral and negatively charged lipids. Illustrative of neutral lipids include phosphatidyl serine and phosphatidyl ethanolamine. In the most preferred embodiment of the invention, the molar ratio of a negatively charged phospholipid to the drug varies from about 0.5 to about 3.0 with a ratio of drug to a neutral phospholipid being from about 1.0 to about 7.0.
- 10  
15  
20

- The pH of the formulation can range from pH 3 to pH 11. The pH of the formulation is adjusted by adding an alkali or an acid as needed. The requisite pH of the formulation is determined by the kind of drug to be entrapped and its solubility in water at ambient temperatures.
- 25

- The liposome formulation may contain other optional ingredients, which include minor amounts of nontoxic, auxiliary substances; such as antioxidants, *e.g.*, butylated hydroxytoluene, alpha-tocopherol and ascorbyl palmitate, ascorbic acid, butylated hydroxyanisole, fumaric acid, malic acid, propyl gallate, sodium ascorbate, and sodium metabisulfite; and pH buffering agents; such as phosphates, glycine and the like. When naturally occurring phospholipids are used, it is recommended that the liposome formulation contain antioxidant to prevent the oxidation of the unsaturated bonds present in the fatty acid chains in the phospholipid.
- 30

- The liposome formulation may contain cryoprotective agent known to be useful in the art of preparing freeze-dried formulations, such as di- or polysaccharides or other bulking agent such as lysine. Further, isotonic agents typically added to maintain isomolarity with body
- 35

fluids may be used. In a preferred embodiment, a di-saccharide or polysaccharide is used and functions both as a cryoprotective agent and as an isotonic agent.

The addition of a disaccharide or polysaccharide provides instantaneous hydration and the largest surface area for depositing a thin film of the drug-lipid complex. This thin film  
5 provides for faster hydration so that, when liposome is initially formed by adding the aqueous phase, the liposomes formed are of a smaller and more uniform particle size. This provides significant advantages in terms of manufacturing ease.

Accordingly, the preferred liposomal composition of the present invention comprises a disaccharide or polysaccharide, in addition to the phospholipids. When present, the  
10 disaccharide or polysaccharide is preferably chosen from among the group consisting of lactose, trehalose, maltose, maltotriose, palatinose, lactulose or sucrose, with lactose or trehalose being preferred. Even more preferably, the liposomes comprise lactose or trehalose.

When present, the di- or polysaccharide is formulated in a preferred ratio of about 10-20 saccharide to 0.5-6.0 phospholipid mixture, respectively, even more preferably at a ratio from  
15 about 10 to 1.5-4.0.

The presence of the disaccharide or polysaccharide in the composition not only tends to yield liposomes having extremely small and narrow particle size ranges, but also provides a liposome composition in which drug, in particular, may be incorporated in an efficient manner, *i.e.*, with an encapsulation efficiency approaching 80-100%. Moreover, liposomes made with a  
20 saccharide typically exhibit improved physical and chemical stability, such that they can retain an incorporated compound without leakage upon prolonged storage, either as a reconstituted liposomal suspension or as a cryodesiccated powder.

The liposomes can be prepared by any of the methods known in the art. (See for example, R. R. C. New, "*Liposomes, a Practical Approach*", pp 1-161, IRL Press, (1990)).

In one of the methods for preparation of liposomes, a solution of a macrolide drug, excipients and lipid in an organic solvent is formed and introduced into a round bottom flask. Suitable solvents include any volatile organic solvent, such as diethyl ether, acetone, methylene chloride, chloroform, piperidine, piperidine-water mixtures, methanol, tert-butanol, dimethyl sulfoxide, N-methyl-2-pyrrolidone, and mixtures thereof. Preferably, the organic solvent is  
30 water-immiscible, such as methylene chloride, but water immiscibility is not required. In any event, the solvent chosen should not only be able to dissolve all of the components of the lipid film, but should also not react with, or otherwise deleteriously affect, these components to any significant degree.

The organic solvent is then removed from the resulting solution to form a dry lipid film  
35 by any known laboratory technique that is not deleterious to the dry lipid film. Preferably, the solvent is removed under a vacuum until all the organic solvent is evaporated. A rotary evaporator is immersed into a thermostated water bath at 40°C, evacuated, and rotated at 60

rpm. The evacuation and rotation is continued until all the solvent has evaporated from the solution and a dry lipid film has deposited on the walls of the flask. The residual solvent is removed by a vacuum pump for a minimum period of twenty four hours. The dry lipid film is then dispersed in an aqueous solution, preferably containing a disaccharide or polysaccharide, or a saline solution. Examples of the useful aqueous solutions used during hydration of the film include sterile water, a calcium- and magnesium-free, phosphate-buffered (pH 7.2-7.4) sodium chloride solution, a 5% dextrose solution, or any other physiologically acceptable aqueous solution of one or more electrolytes. Preferably, however, the aqueous solution is sterile. The volume of the aqueous solution used during hydration can vary greatly, but should be higher than about 35% and less than about 95% of the total volume. The flask is rotated at 60 rpm at room temperature until all the lipid film has been removed from the wall of the flask. The lipid dispersion is then allowed to stand for additional two hours at room temperature in order to complete the swelling process. The coarse liposome thus formed incorporates a drug-lipid complex. The hydration step should take place at a temperature that does not exceed about 40°C, preferably, at room temperature or lower.

The particle size of the coarse liposome obtained is homogenized by passing the dispersion through a high-speed agitation devices; such as "Microfluidizer", for example, a Microfluidics™ Model 110F; a sonicator; a high-shear mixer; a homogenizer; or a standard laboratory shaker. Preferably, a high pressure device such as a Microfluidizer™ is used for agitation. In microfluidization, a great amount of heat is generated during the short period of time during which the fluid passes through a high pressure interaction chamber. For this reason, the homogenization temperature is cooled down to a temperature no greater than 40°C after the composition passes through the zone of maximum agitation, *e.g.*, the interaction chamber of a Microfluidizer™ device. The homogenization temperature should preferably be room temperature or lower. While the pressure used in such high pressure devices is not critical, pressures from about 10,000 to about 16,000 psi are not uncommon. It is crucial that one obtains vesicles which are of right size and structure, and entrap materials with high efficiency and in such a way that materials do not leak out of the liposome once formed.

The pH of the formulation is adjusted to a desired level by adding an alkali or an acid as the case may be.

The liposome dispersion so obtained is aseptically filtered through a 0.2 µm filter using a syringe pump and the post filtrate weighed to determine filterability. The filtration potency of the prefiltrate and postfiltrate can be determined using High Performance Liquid Chromatography (HPLC). The particle size distribution of the vesicles can be determined using a NICOMP (Model 370) submicron particle analyzer or other conventional particle size measuring techniques.

The liposomal compositions of the invention provide liposomes of a sufficiently small particle size such that the aseptic filtration of the composition through a 0.2  $\mu\text{m}$  hydrophilic filter can be accomplished efficiently. The particle size of the liposome can vary from about 10 nm to about 25  $\mu\text{m}$ . A particularly preferred size range is below about 300 nm, more preferably below from about 250 nm. Most preferably, the particle size is below about 220 nm. The liposome particles can be uniform in size or can be bimodes or multiple modes.

Filterability can be tested by passing a liposome composition through a Microfluidizer™ five times and withdrawing a sample with a syringe. The syringe is connected to a 0.2  $\mu\text{m}$  hydrophilic filter and then placed in a syringe pump. The constant rate of piston movement is set at 10 ml/min, and filtrate is collected until the filter becomes blocked by large aggregates of liposome. The volume or weight of the filtrate is then measured and recorded in terms of ml/cm<sup>2</sup> or g/cm<sup>2</sup>, with a square centimeter being the effective filtration area. Thus, filterability for the purpose of the invention is defined as the maximum volume or weight of liposomal composition that can be filtered through 0.2  $\mu\text{m}$  filter.

The liposome compositions of the invention are typically administered parenterally. Injection may be intravenous, subcutaneous, intramuscular, intrathecal, intraperitoneal, and any other parenteral administrations.

The quantity of the liposome formulation to be administered depends on the choice of active drug, the condition to be treated, the mode of administration, the individual subject, and the judgment of the practitioner. Generally speaking, however, dose of active drug in the range of 0.05-50 mg/kg may be needed. The foregoing range is, of course, merely suggestive, as the number of variables in regard to an individual treatment regime is large. Therefore, considerable excursions from these recommended values are expected.

The packing of bilayers in liposome vesicle is very tight with higher glass transition temperature than that of micelles and emulsion, and, therefore, the liposome vesicle is more rigid and less dynamic than micelles and emulsions. The physical barriers created by liposome of the invention, especially multibilayered liposome vesicles, are effective against rapid movement of the drug therefrom at the injection site after such infusion, thereby reducing the concomitant pain.

#### Pain Evaluation Using Rat Tail Vein Infusion Pain Model

The effectiveness of the formulation in reducing pain is tested using a Rat Tail Vein Infusion motor response model. This model is based on the assumption that the movement of a rat during intravenous infusion will be proportional to the pain caused by the infusion. The rat (male Spargue Dawley, 325 to 375 g) is placed in a restraining cage mounted on springs. Multiple test substances are infused successively in a desired sequence, through a butterfly needle inserted in the lateral tail vein of a rat. Movement of the cage is monitored by an



accelerometer mounted at the base of the cage. The accelerometer signal is transmitted to a computer for data display and storage.

The following Examples illustrate the invention and are not to be construed as limiting of the invention.

### Example 1

The formulations of Example 1(a) through 1(f), containing various ingredients in the amounts set forth below in Table 1, were prepared by the following general method.

**Table 1**

Ingredients	Amount/25 ml	% (w/v)
Clarithromycin	125 mg	0.5
Dimyristoyl Phosphatidylcholine (DMPC)	793 mg	3.2
Phosphatidylglycerol (PG)*	66.8-401 mg	1.6
Butylated Hydroxytoluene (BHT)	1.25 mg	0.005
Lactose, NF, Injectable	2500 mg	10
Water for Injection	q.s. 25 ml	N/A

\* Amount of PG varied, see Table 2.

A drug-lipid organic solution was prepared by dissolving PG, DMPC, clarithromycin, and BHT in 25 ml of methylene chloride. The lipid solution is introduced in a round-bottomed flask. The flask is attached to a rotary evaporator, and immersed into in a thermostated water bath at 4 °C. The flask is rotated at 60 rpm and vacuum applied until all the solvent has evaporated from the solution and a dry lipid film has deposited on the walls of the flask.

The trace residual solvent is removed by applying vacuum for a minimum period of 24 hours. The flask is then flushed with nitrogen and lactose solution added. The flask is rotated (60 rpm) at room temperature until all the lipid film has been removed from the wall of the flask. The lipid dispersion is allowed to stand for an additional 2 hours at room temperature in order to complete the swelling process.

The particle size of the coarse liposome is reduced by passing the liposomal solution through a Microfluidizer™ five times. The pH is adjusted to an appropriate value by adding an alkali or an acid. The liposomal dispersion is filtered through a 0.2 µm hydrophilic filter using a syringe pump and post filtrate weighed to determine filterability. The filtration potency of prefiltrate and postfiltrate is determined using an HPLC method. The particle size distribution is measured using a NICOMP submicron particle analyzer (Model 370).

Table 2 below sets forth the various parameters for the formulations of Example 1(a) through 1(f). The amount of clarithromycin contained in the formulation is 5 mg/ml of the dispersion. The particle size distribution of liposome is bimodal.

**Table 2**

Example 1	pH	PG (MR *)	Filtration Potency (%)	Filterability (g/cm <sup>2</sup> )	Mean Particle Size nm**
a	8	0.5	45.9	1.28	110/11
b	8	1	70.3	0.46	37/10
c	8	3	97.8	2.78	77/5
d	10	0.5	19.5	0.24	92/14
e	10	1	17.5	0.24	104/17
f	10	3	39.7	0.12	41/6

\* means the molar ratio of PG to clarithromycin

\*\* means Nicomp distribution: 2 modes

It can be seen from Table 2 that at pH 8, post-filtration potency increased with increasing PG MR. Nearly 100% of the liposomes with 3 PG MR passed through a 0.2 micron filter. In contrast, around 50% of the 0.5 PG MR liposomes and around 70% of the 1PG MR liposomes passed through the 0.2 micron filter. These results indicate that PG effectively increases the entrapment of clarithromycin into the lipid bilayer at pH 8. Without being bound by theory, it is believed that this effect may be caused by the electrostatic attraction of the partial positively charged clarithromycin and the negatively charged PG.

At pH 10, the post-filtration potencies were lower than the corresponding pH 8 values for all PG MRs. Increases in MR did not show a significant increase in post-filtration potency. At 3 PG MR, only around 40% of the clarithromycin liposomes passed through the 0.2 micron filter. The negatively charged PG does not have a significant effect on the entrapment of clarithromycin into the lipid bilayer. The diminished effect of PG on entrapment may be due to the lack of electrostatic attraction between PG and clarithromycin free base at pH 10. The untrapped clarithromycin may cause aggregation of liposomes and/or precipitate due to its insoluble nature. Consequently, these large particles may clog the filter and result in low potency.

### Example 2

A liposomal formulation was prepared in accordance with the method described in Example 1. Table 3 sets forth the amounts of the ingredients used to prepare the formulation. The amount of clarithromycin in the formulation is 3.5 mg/ml of the dispersion. The

concentration (3.5 mg/ml) was determined using an HPLC method. The pH of the suspension is 8.0 with the filtration potency of 100% and filterability of 0.31g/cm<sup>2</sup>. The particle size distribution of liposome is bimodal having particle size of 83.4/19.9 nm.

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**Table 3**

Ingredients	Amount/35 ml	% (w/v)
Clarithromycin	125 mg	0.35
Dimyristoyl Phosphatidylcholine (DMPC)	793 mg	2.3
Dimyristoyl Phosphatidylcholine (DMPA)	308 mg	0.9
Butylated Hydroxytoluene (BHT)	1.25 mg	0.005
Lactose, NF, Injectable	3,500 mg	10
Water for Injection	q.s. 35 ml	N/A*

\* means not applicable.

**Example 3**

10 A liposomal formulation comprising ABT-229 was prepared in accordance with the method described in Example 1. Table 4 sets forth the amounts of the ingredients used to prepare the formulation. The amount of ABT-229 in the formulation is 2.5 mg/ml of the dispersion. The concentration (2.5 mg/ml) was determined using an HPLC method. The pH of the suspension is 8.0 with the filtration potency of 97.9% and filterability of

15 >4.0 g/cm<sup>2</sup>. The particle size distribution of liposome is trimodal having particle size of 7.9/62.5/296.4 nm.

**Table 4**

Ingredients	Amount/25 ml	% (w/v)
ABT-229	125 mg	0.25
Dimyristoyl Phosphatidylcholine (DMPC)	850 mg	1.7
Phosphatidylglycerol (PG)	430 mg	0.86
Butylated Hydroxytoluene (BHT)	2.5 mg	0.005
Lactose, NF, Injectable	5,000 mg	10
Water for Injection	q.s. 50 ml	N/A*

20 \* means not applicable.

**Example 4**

A liposomal formulation comprising ABT-773 was prepared in accordance with the method described in Example 1. Table 5 sets forth the amounts of the ingredients used to prepare the formulation. The amount of ABT-773 in the formulation is 5.0 mg/ml of the dispersion. The concentration (5.0 mg/ml) was determined using an HPLC method. The pH of the suspension is 9.0 with the filtration potency of 103.4% and filterability of >4.0 g/cm<sup>2</sup>. The particle size distribution of liposome is unimodal having particle size of 15.4 nm.

**Table 5**

Ingredients	Amount/50ml	% (w/v)
ABT-773	250 mg	0.5
Dimyristoyl Phosphatidylcholine (DMPC)	1550 mg	3.1
Phosphatidylglycerol (PG)	783 mg	1.57
Butylated Hydroxytoluene (BHT)	2.5 mg	0.005
Lactose, NF, Injectable	5,000 mg	10
Water for Injection	q.s. 50 ml	N/A*

\* means not applicable.

**Example 5**

This example illustrates the evaluation of pain reducing properties of the liposomal formulations using Rat Tail Vein Infusion Pain Model. Rats are initially acclimated to the restraining cage for a period of thirty minutes, one to five days prior to infusion of the test formulation. The formulations of the above examples are then infused by inserting a 23 gauge butterfly needle in the lateral tail vein of the rats. The needle is connected by tubing to a syringe pump and formulations are infused at 0.1 ml/minute. Infusion of each formulation is preceded and followed by saline. Movement of the cage is monitored by an accelerometer mounted at the base of the cage. The accelerometer signal is transmitted to a computer for data display and storage.

Figure 1 illustrates the results of infusing 5 mg/ml clarithromycin lactobionic acid solution preceded and followed by saline. At this concentration, clarithromycin solution leads to a definite pain response.

Figure 2 illustrates the results of the pain producing property of the formulation of the invention of Example 1 as compared to placebo liposomes, saline, and clarithromycin solution (5 mg/ml). The results are obtained by sequentially infusing saline, placebo liposomes, saline,

clarithromycin liposomes of Example 1, saline and clarithromycin solution using same individual rat throughout the experiment, thereby eliminating uncertainty caused by inter animal variability. The results clearly indicate that the rat showed no response to the placebo liposomes or the clarithromycin loaded liposomes. The pain response to the clarithromycin solution is typical for 5 mg/ml and similar to the results shown in Figure 1.

Figure 3 illustrates the results of an experiment designed to compare the pain producing property of the formulation of Example 2 and free clarithromycin at 3.5 mg/ml. The results are obtained by sequentially infusing saline, placebo liposomes, saline, clarithromycin liposomes of Example 2, saline, clarithromycin solution, and saline using same individual rat throughout the experiment, thereby eliminating uncertainty caused by inter animal variability. The results clearly indicate that the rat showed no response to the placebo liposomes or the clarithromycin loaded liposomes. The pain response to clarithromycin solution is typical for 3.5 mg/ml and similar to the results obtained in Figure 1.

Figures 4 illustrates the results of the pain producing property of the formulation of the invention of Example 3. The results are obtained by sequentially infusing saline, placebo liposome, saline, ABT-229 liposome formulation of Example 3, saline, placebo IV (lactobionic acid solution), saline, ABT-229 solution IV, and saline using Rat Tail Vein Infusion Pain Model using same individual rat throughout the experiment, thereby eliminating uncertainty caused by inter animal variability. The results clearly indicate that the rat showed no response to the placebo liposomes, placebo IV or the ABT-229 loaded liposomes but showed the pain response to the ABT-229 solution IV. In this Example, placebo IV was infused to demonstrate that the pain response is associated with ABT-229 and not the lactobionic acid itself.

Figures 5 illustrates the results of the pain producing property of the formulation of the invention of Example 4. The results are obtained by sequentially infusing saline, placebo liposome, saline, ABT-773 liposome formulation of Example 4, saline, placebo V (lactobionic acid solution), saline, ABT-773 solution V, and saline using same individual rat throughout the experiment, thereby eliminating uncertainty caused by inter animal variability. The results clearly indicate that the rat showed no response to the placebo liposomes, placebo V or the ABT-773 loaded liposomes but showed the pain response to the ABT-773 solution. In this Example, placebo V was infused to demonstrate that the pain response is associated with ABT-773 and not the lactobionic acid itself.

These Figures clearly illustrate that entrapping a macrolide in the liposomes effectively reduced the painful nature of clarithromycin on injection to a level that was not detectable using the Rat Tail Vein Infusion Pain model.

## WHAT IS CLAIMED IS:

1. A pain-reducing parenteral formulation comprising a macrolide drug selected from the group consisting of derivatives of erythromycins A, B, C, and D; clarithromycin; azithromycin; dirithromycin; josamycin; midecamycin; kitasamycin; roxithromycin; rokitamycin; oleandomycin; miocamycin; flurithromycin; rosaramicin; 8,9-anhydro-4"-deoxy-3'-N-desmethyl-3'-N-ethylerythromycin B 6,9-hemiacetal; 8,9-anhydro-4"-deoxy-3'-N-desmethyl-3'-N-ethylerythromycin A 6,9-hemiacetal; and 11-amino-11-deoxy-3-oxo-5-O-desosaminyl-6-O-[1'-3'-quinolyl-2'-propenyl]-erythronolide A 11,12-cyclic carbamate entrapped in a liposome vesicle comprising a lipid, wherein at least one lipid is negatively charged, and the molar ratio of the drug to the lipid ranges from about 1: 2 to about 1:100.
2. The formulation of Claim 1, wherein the lipid is selected from the group consisting of phospholipids, fatty acids, double chain secondary amines and cholesterol.
3. The formulation of Claim 2, wherein the lipid is selected from the group consisting of phosphatidyl choline, sphingomyelin, phosphatidyl ethanolamine, phosphatidyl-inositol, phosphatidyl serine, ether-linked phosphatidyl choline, dimyristoyl phosphatidyl choline, egg phosphatidyl glycerol, dimyristoyl phosphatidic acid, and distearoyl lecithin.
4. The formulation of Claim 3, wherein the molar ratio of the negatively charged lipid to the drug in the vesicle varies from about 0.5 to about 3.0, and the molar ratio of the drug to the neutral lipid varies from 1.0 to 7.0.
5. The formulation of Claim 1, wherein the pH of the formulation varies from about 3.0 to about 11.0.
6. The formulation of Claim 1, wherein the average size of the liposome vesicles ranges from about 10 nm to about 25  $\mu$ m.
7. The formulation of Claim 1, further comprising antioxidants.
8. The formulation of Claim 1, wherein the antioxidant is selected from the group consisting of butylated hydroxytoluene, alpha-tocopherol, ascorbyl palmitate, ascorbic acid, butylated hydroxyanisole, fumaric acid, malic acid, propyl gallate, sodium ascorbate, and sodium metabisulfite.

9. The formulation of Claim 8, wherein the macrolide is selected from the group consisting of derivatives of erythromycins A, B, C, and D; clarithromycin; azithromycin; roxithromycin; 8,9-anhydro-4"-deoxy-3'-N-desmethyl-3'-N-ethylerythromycin B 6,9-hemiacetal; 8,9-anhydro-4"-deoxy-3'-N-desmethyl-3'-N-ethylerythromycin A 6,9-hemiacetal; and 11-amino-11-deoxy-3-oxo-5-O-desosaminyl-6-O-[1'-3'-quinolyl-2'-propenyl]-erythronolide A 11,12-cyclic carbamate.
10. The formulation of Claim 9 further comprising a disaccharide or polysaccharide.
11. The formulation of Claim 10, wherein the disaccharide or polysaccharide is selected from the group consisting of lactose, trehalose, maltose, maltotriose, palatinose, lactulose and sucrose.
12. The formulation of Claim 11, wherein the macrolide is clarithromycin.
13. A method of reducing injection site pain caused by a macrolide drug comprising administering a parenteral formulation comprising the macrolide drug entrapped in a liposome vesicle.
14. The method according to Claim 13, wherein the liposome vesicle comprises lipids selected from the group consisting of phospholipids, fatty acids, double chain secondary amines and cholesterol.
15. The method according to Claim 14, wherein the lipid is selected from the group consisting of phosphatidyl choline, sphingomyelin, phosphatidyl ethanolamine, phosphatidyl-inositol, phosphatidyl serine, ether-linked phosphatidyl choline, dimyristoyl phosphatidyl choline, egg phosphatidyl glycerol, dimyristoyl phosphatidic acid, dipalmitoyl lecithin, and distearoyl lecithin.
16. The method according to Claim 15, wherein the molar ratio of a negatively charged lipid to the drug in the vesicle varies from about 0.5 to about 3.0, and the molar ratio of the drug to a neutral lipid varies from about 1.0 to about 7.0.
17. The method according to Claim 16, wherein the pH of the formulation varies from about 3.0 to about 11.0.

18. The method according to Claim 13, wherein the average size of the liposome vesicle varies from about 10 nm to about 25  $\mu$ m.

19. The method according to Claim 13, wherein the formulation further comprises  
5 antioxidants.

20. The method according to Claim 19, wherein the antioxidant is selected from the group consisting of butylated hydroxytoluene, alpha-tocopherol, ascorbyl palmitate, ascorbic acid, butylated hydroxyanisole, fumaric acid, malic acid, propyl gallate, sodium ascorbate, and  
10 sodium metabisulfite.

21. The method according to Claim 20, wherein the macrolide drug is selected from the group consisting of derivatives of erythromycins A, B, C, and D; clarithromycin; azithromycin; roxithromycin; 8,9-anhydro-4"-deoxy-3'-N-desmethyl-3'-N-ethylerythromycin  
15 B 6,9-hemiacetal; 8,9-anhydro-4"-deoxy-3'-N-desmethyl-3'-N-ethylerythromycin A 6,9-hemiacetal; and 11-amino-11-deoxy-3-oxo-5-O-desosaminyl-6-O-[1'-3'-quinolyl-2'-propenyl]-erythronolide A 11,12-cyclic carbamate.

22. The method according to Claim 21, wherein the formulation further comprises a  
20 disaccharide or polysaccharide.

23. The method according to Claim 22, wherein the disaccharide or polysaccharide is selected from the group consisting of lactose, trehalose, maltose, maltotriose, palatinose, lactulose and sucrose.

24. The method according to Claim 23, wherein the macrolide is clarithromycin.  
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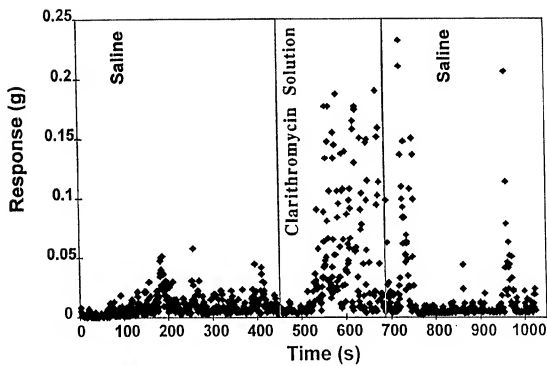


FIG. 1

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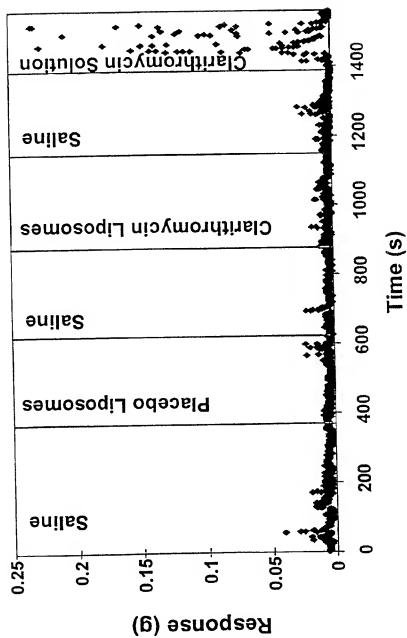


FIG. 2

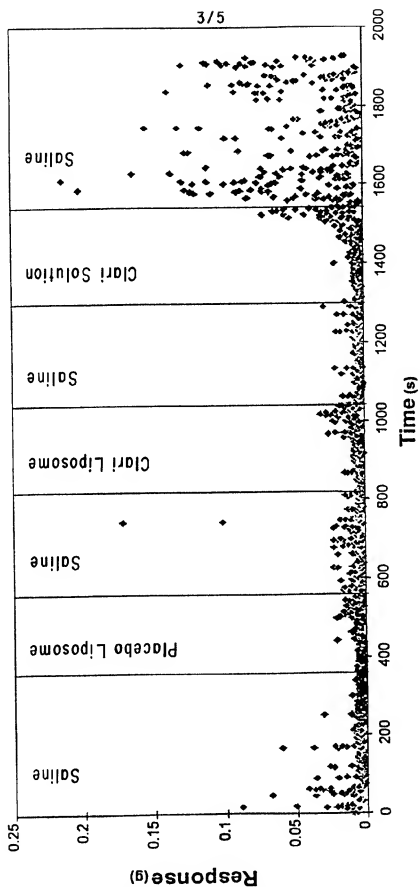


FIG. 3

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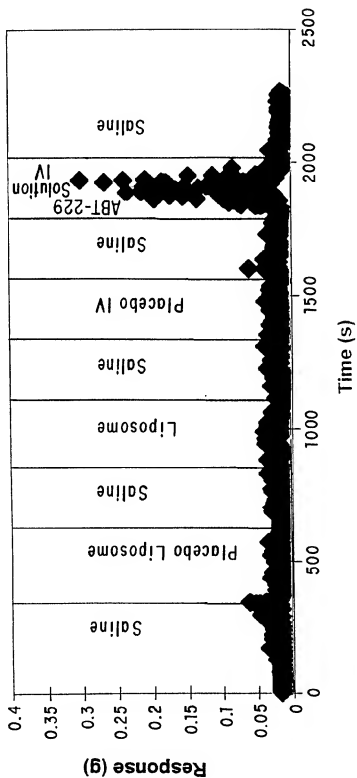


FIG. 4

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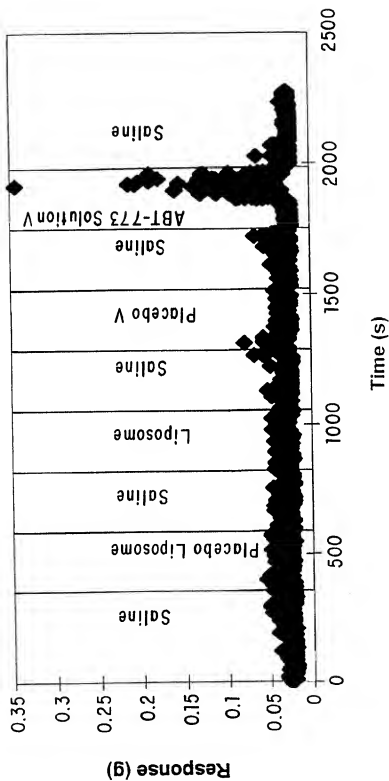


FIG. 5

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 98/01430

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 A61K9/127 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	L. STUHNE-SEKALEC ET AL.: "Liposomes as carriers of macrolides: preferential association of erythromycin A and azithromycin with liposomes..." JOURNAL OF MICROENCAPSULATION, vol. 8, no. 2, April 1991, LONDON, GB, pages 171-183, XP000216616 see abstract see page 172 see Discussion see table 2	1-3,5,6
Y	---	7-12
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is used to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "I" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "S" document member of the same patent family

Date of the actual completion of the international search

19 May 1998

Date of mailing of the international search report

01.07.1998

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## INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 98/01430

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DATABASE WPI Week 9439 Derwent Publications Ltd., London, GB; AN 94-313609 XP002065383 & JP 06 239 734 A (KANEBO LTD.) , 30 August 1994 see abstract ---	7-9
Y	WO 87 01933 A (LIPOsome TECHNOLOGY, INC.) 9 April 1987 see claims 1,3-8,11,14-16 ---	10,11
X	CYPRIAN O. ONYEJI ET AL.: "Efficacies of liposome-encapsulated clarithromycin and ofloxacin against ..." ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, vol. 38, no. 3, March 1994, pages 523-527, XP002065380 see page 523 ---	1-3
Y	---	12
X	SHYAMALA C. JAYARAMAN ET AL.: "Topical delivery of erythromycin from various formulations..." JOURNAL OF PHARMACEUTICAL SCIENCES, vol. 85, no. 10, October 1996, pages 1082-1084, XP002065381 see abstract ---	1,2,5
X	YU-KYOUNG OH ET AL.: "Formulation and efficacy of liposome-encapsulated antibiotics..." ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, vol. 39, no. 9, September 1995, pages 2104-2111, XP002065382 see abstract ---	1,2
A	WO 90 04961 A (BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM) 17 May 1990 see claims 1-16 ---	1-5,9
A	WO 89 03208 A (BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM) 20 April 1989 see page 11, line 15 - page 15, line 27 ---	1-5,9
A	WO 88 06440 A (BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM) 7 September 1988 see page 9, line 1 - line 23 see page 10, line 22 - line 25 see page 11, line 14 - line 26 see page 12, line 8 - line 12 see page 15, line 21 - line 23 ---	1-5,9
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## INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 98/01430

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 3 105 793 A (MERVYN JOSEPH LOBEL) 1 October 1963 see column 1, line 15 - line 24 see column 1, line 51 - line 54 see claims 1-4 -----	13-24



## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 98/01430**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Although claims 13-24 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/01430

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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WO 9004961 A	17-05-1990	US 4999199 A AU 4509389 A	12-03-1991 28-05-1990
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WO 8806440 A	07-09-1988	US 4812312 A AU 1491788 A DE 3873528 A EP 0348431 A JP 2502459 T JP 2703594 B	14-03-1989 26-09-1988 10-09-1992 03-01-1990 09-08-1990 26-01-1998
US 3105793 A	01-10-1963	NONE	